Supplemental Figures Legends

Figure S1: Growth rates of NCI-H526 (A, left) and DMS114 (B, left) cells stably expressing either control shRNA or two independent shRNAs targeting LSD1 following 4, 7, or 14 days post-infection with lentiviral vector. Knockdown of LSD1 was assessed by western blot at day 4 post-infection in NCI-H526 (A, right) and DMS114 (B, right) cells. Lentiviral shRNA vectors pRSI12-U6-(sh)-UbiC-TagRFP-2A-Puro were purchased from Cellecta with targeting sequences for non-targeting (CAACAAGATGAAGAGCACCAA), LSD1 shRNA #1 (CCAACAATTAGAAGCACCTTA) and LSD1 shRNA #2 (AGGAAGGCTCTTCTAGCAATA). Each shRNA lentiviral expression construct was packaged with lentiviral packaging mix (Thermo Scientific) in 293T cells according to manufacturer's instructions. Cell growth was monitored by CellTiter-Glo Luminescent Cell Viability Assay (Promega) at day 7 and day 14 from experimental triplicates.

Figure S2: Structures and profile of GSK690 (R)-4-(5-(pyrrolidin-3-ylmethoxy)-2-(p-tolyl)pyridin-3-yl)benzonitrile and OG-86 (1S,2R)-N-((2-methoxypyridin-3-yl)methyl)-2-phenylcyclopropan-1-amine compounds based off publicly available data.

Figure S3: Long-term proliferation assays for NCI-H1417 (A), NCI-H187 (B), NCI-H889 (C), and DMS-114 (D) cells treated with vehicle (DMSO), 0.3 μM or 1.0 μM GSK690, or 0.3 μM or 1.0 μM OG-86. Cell number was calculated from experimental triplicates by cell counting at indicated time points.

Figure S4: Gene expression (Log2) and copy number of MYC, MYCN, and MYCL in GSK690 sensitive and resistant SCLC cell lines. Expression and copy number were determined from CCLE data for cell lines used in analysis. Significance was calculated using an unpaired t-test and samples with $p<0.05$ are indicated.

Figure S5: (A) Principal component analysis of cell line gene expression derived from CCLE cell line data showing segregation of GSK690 sensitive models (green) and resistant models (red) on PC1 vs. PC2. (B) Venn diagram showing overlap of DE gene signature from Figure 3A with previously published NE or ML signature genes (17). (C) Pathway analysis of genes altered in expression (FDR < 0.05, fold change >=2) by GSK690 treatment utilizing MSigDB pathway databases. Top 10 enriched pathways are shown with the p-value cutoff of 0.05.

Figure S6: Western Blot analysis of neuroendocrine and mesenchymal protein levels using indicated antibodies in SCLC cells lines treated with DMSO or 0.3 μM GSK690 for 14 days.

Figure S7: (A) Left: cluster number selection within UMAP using resolution = 0.15. To optimize single cell clustering, we tuned the “resolution” parameter in Seurat, and selected resolution = 0.15 by visual inspection and the presence of robust differential markers between clusters. Right: cell number change in each cluster depicted as percentage of total cells in each cluster before and after GSK690 treatment. (B) Expression of ASCL1 or GRP in cluster 2 in UMAP or as violin plots following treatment with vehicle (DMSO) or 0.3 μM GSK690 for 21 days.

Figure S8: (A) Top 10 uniquely expressed genes in each single cell RNA-seq cluster. The cluster specific genes were identified by performing DE analyses between each cluster with all the rest of clusters. DE cutoff is FDR < 5% and logFC > 0.25. (B) For differential pathway analysis, a hypergeometric test with FDR correction was applied to cluster specific differential genes using the MSigDB data base. NE (8 genes) or EMT (76 genes) gene score were calculated as the mean expression value of the detected gene sets in each single cell, and two sample t-test was performed to calculate the

significance between DMSO and treatment. Pathways analysis showed top 10 up-regulated pathways within each cluster following treatment of 0.3 μM GSK690 for 21 days. P-value cutoff = 0.05. Cluster 3 and 4 did not have any significant enrichment.

Figure S9: Cell viability and gene expression of neuroendocrine and mesenchymal markers in NCI-H526 and NCI-H69 cells pre-treated with 0.3 μM GSK690 for 14 days and following 7-day washout. NCI-H526 and NCI-H69 cells were treated in triplicate for 14 days with DMSO or 0.3 μM GSK690. Every 3 or 4 days, adherent and suspension cells were collected and 2 ml of cells was transferred to 10cm2 dishes for subsequent culture. 8 ml of fresh cell culture medium was added to each plate and drug was added at proper concentrations to each 10 cm dish. After removal of drug for 7 days, remaining cells from the previously pre-treated population with DMSO or 0.3 μM GSK690 were plated at similar concentrations in triplicate. Cells were then exposed to DMSO, 0.1 μM, 0.3 μM, or 1.0 μM of GSK690 and counted at 7, 10, and 14 days following re-exposure to drug. At each time-point, three aliquots of 1 ml of cells was collected in 1.5 ml microcentrifuge tube, concentrated by centrifugation to a 100 μl volume and added to a 96 well plate. Relative cell numbers were determined by cell titer-glo reagent (Promega). Aliquots were also removed at several indicated time points for RNA purification using RNeasy kit (Qiagen). Gene expression of SYP, GRP, NCAM, CHGA, OVOL2, ASCL1, CHD1, CHD2, SNA1, ZEB1, VIM, and MYC were assessed by qRT-PCR using indicated taqman gene expression assays (Applied Biosystems). Expression of each sample was normalized to GAPDH and delta-Ct values calculated from fold change relative to DMSO at each time point.

Figure S10: (A) Representative ATAC-seq profiles of biological replicates from day 0, day 21 DMSO or 0.3 μM GSK690 treatment. (B) Percentage of ATAC-seq peaks in cells treated with GSK690, separated into promoter (<±3 kb transcription start site [TSS]) and distal regions (>±3 kb TSS).

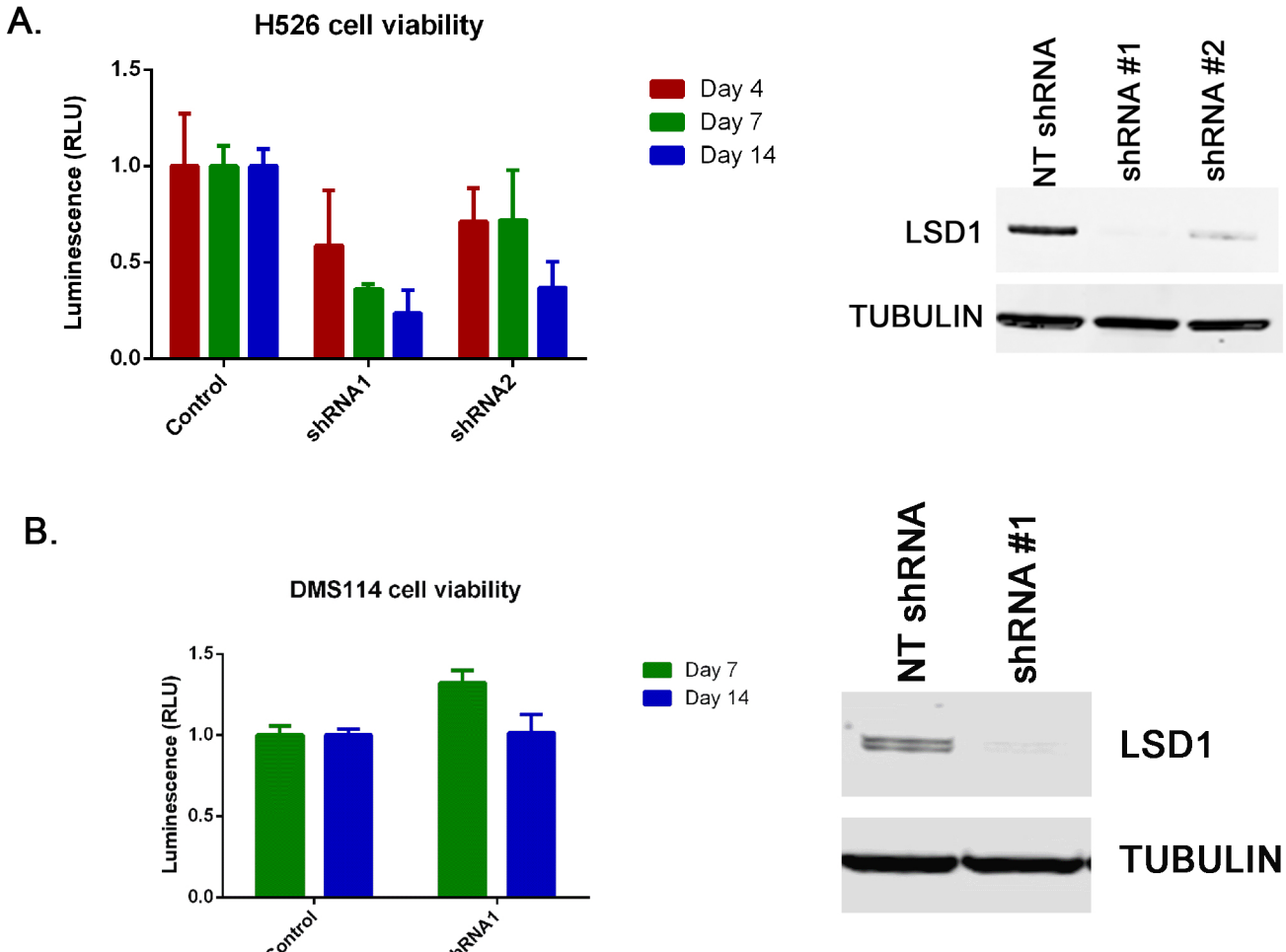
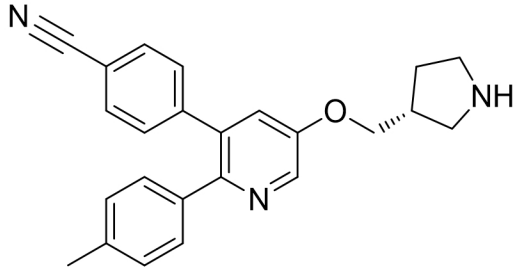

A.
H526 cell viability
Luminescence (RLU)
1.5
1.0
0.5
0.0
Control
shRNA1
shRNA2
Day 4
Day 7
Day 14
NT shRNA
shRNA #1
shRNA #2
LSD1
TUBULIN
B.
DMS114 cell viability
Luminescence (RLU)
1.5
1.0
0.5
0.0
Control
shRNA1
Day 7
Day 14
NT shRNA
shRNA #1
LSD1
TUBULIN

Figure S1

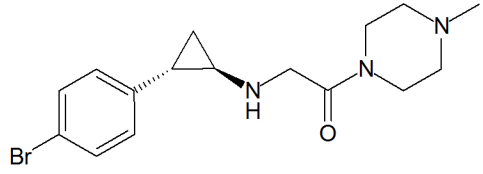
GSK690



GSK690
LSD1/CoREST Ki = 4 nM
Cell/Gene Exp IC50 = 308 nM

Selective vs. LSD2, MAO-A, MAO-B
Competitive w/ H3K4Me2 peptide
Reversible

Oryzon OG-86



OG-86
LSD1 IC50 (uM) - HRP Assay = 0.047

Selective vs. LSD2, MAO-A, MAO-B
Irreversible

Figure S2

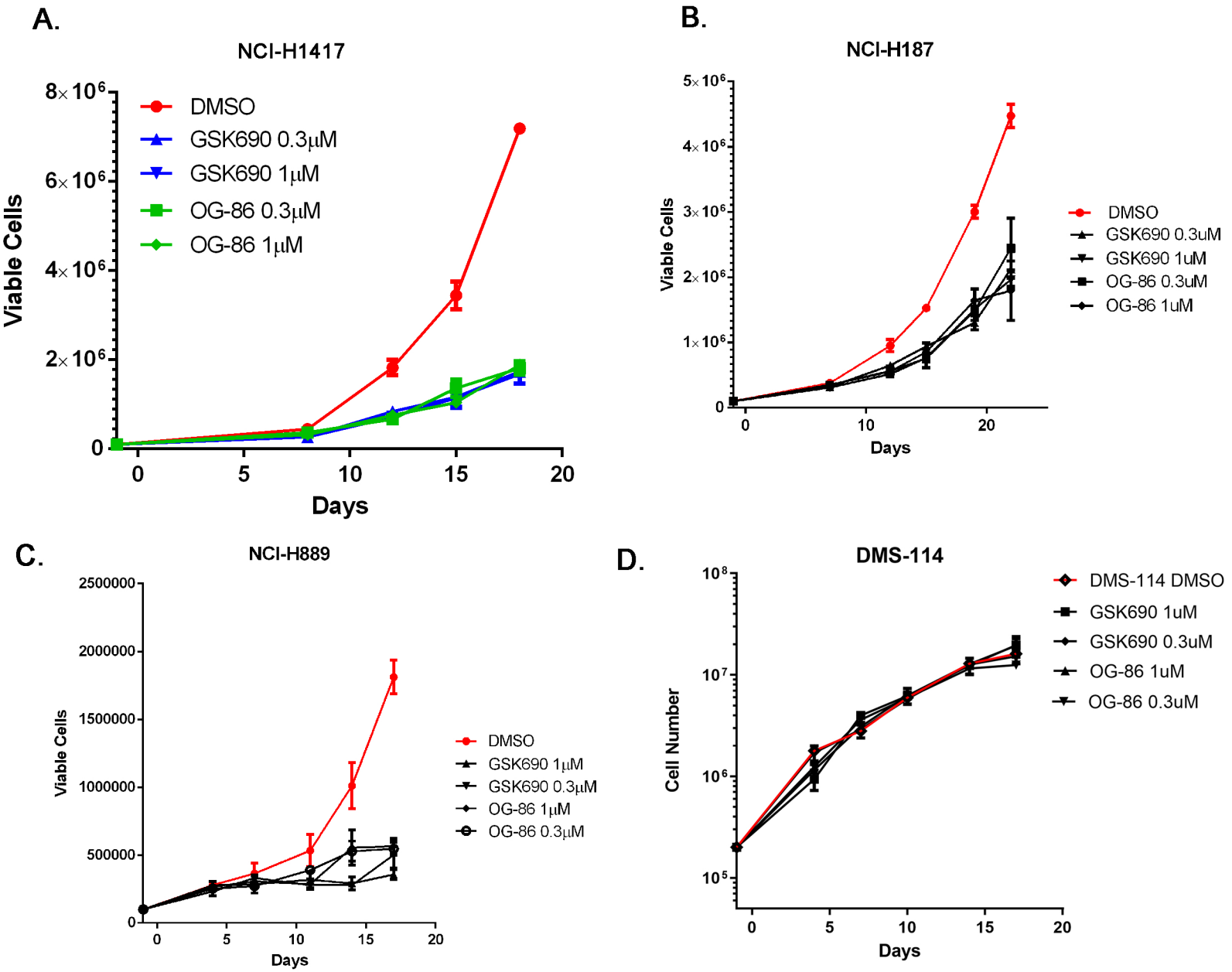
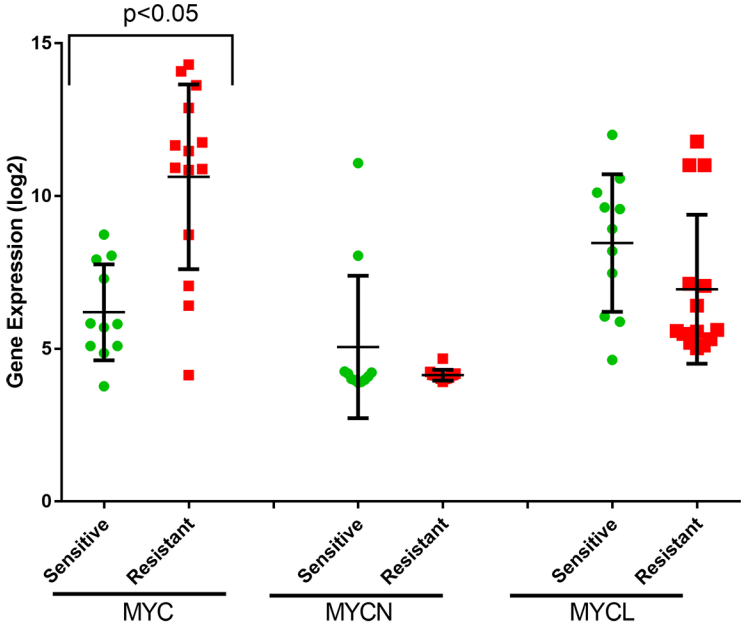

A.
NCI-H1417
Viable Cells
8×10^6
6×10^6
4×10^6
2×10^6
0
0
5
10
15
20
Days
DMSO
GSK690 0.3μM
GSK690 1μM
OG-86 0.3μM
OG-86 1μM
B.
NCI-H187
Viable Cells
5×10^6
4×10^6
3×10^6
2×10^6
1×10^6
0
0
10
20
Days
DMSO
GSK690 0.3uM
GSK690 1uM
OG-86 0.3uM
OG-86 1uM
C.
NCI-H889
Viable Cells
2500000
2000000
1500000
1000000
500000
0
0
5
10
15
20
Days
DMSO
GSK690 1μM
GSK690 0.3μM
OG-86 1μM
OG-86 0.3μM
D.
DMS-114
Cell Number
10^8
10^7
10^6
10^5
0
5
10
15
20
Days
DMS-114 DMSO
GSK690 1uM
GSK690 0.3uM
OG-86 1uM
OG-86 0.3uM

Figure S3


p<0.05
Gene Expression (log2)
15
10
5
0
Sensitive
Resistant
MYC
MYCN
MYCL

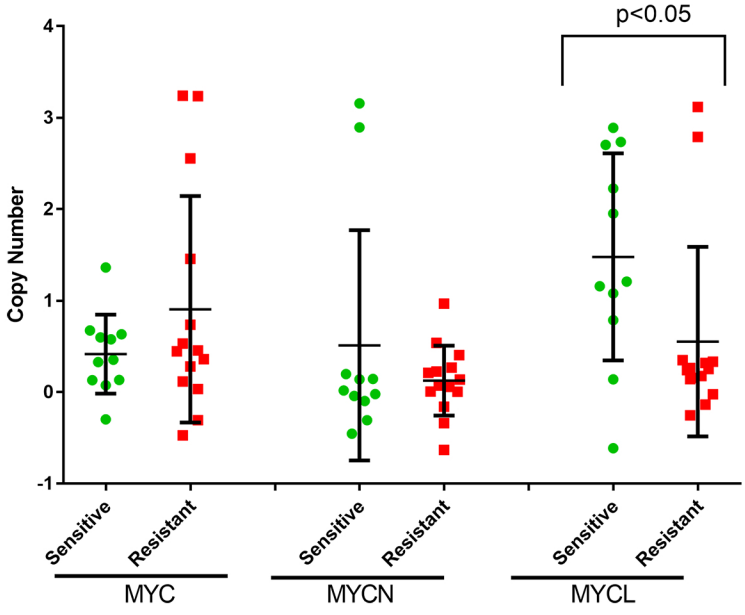

p<0.05
Copy Number
4
3
2
1
0
-1
Sensitive
Resistant
MYC
MYCN
MYCL

Figure S4

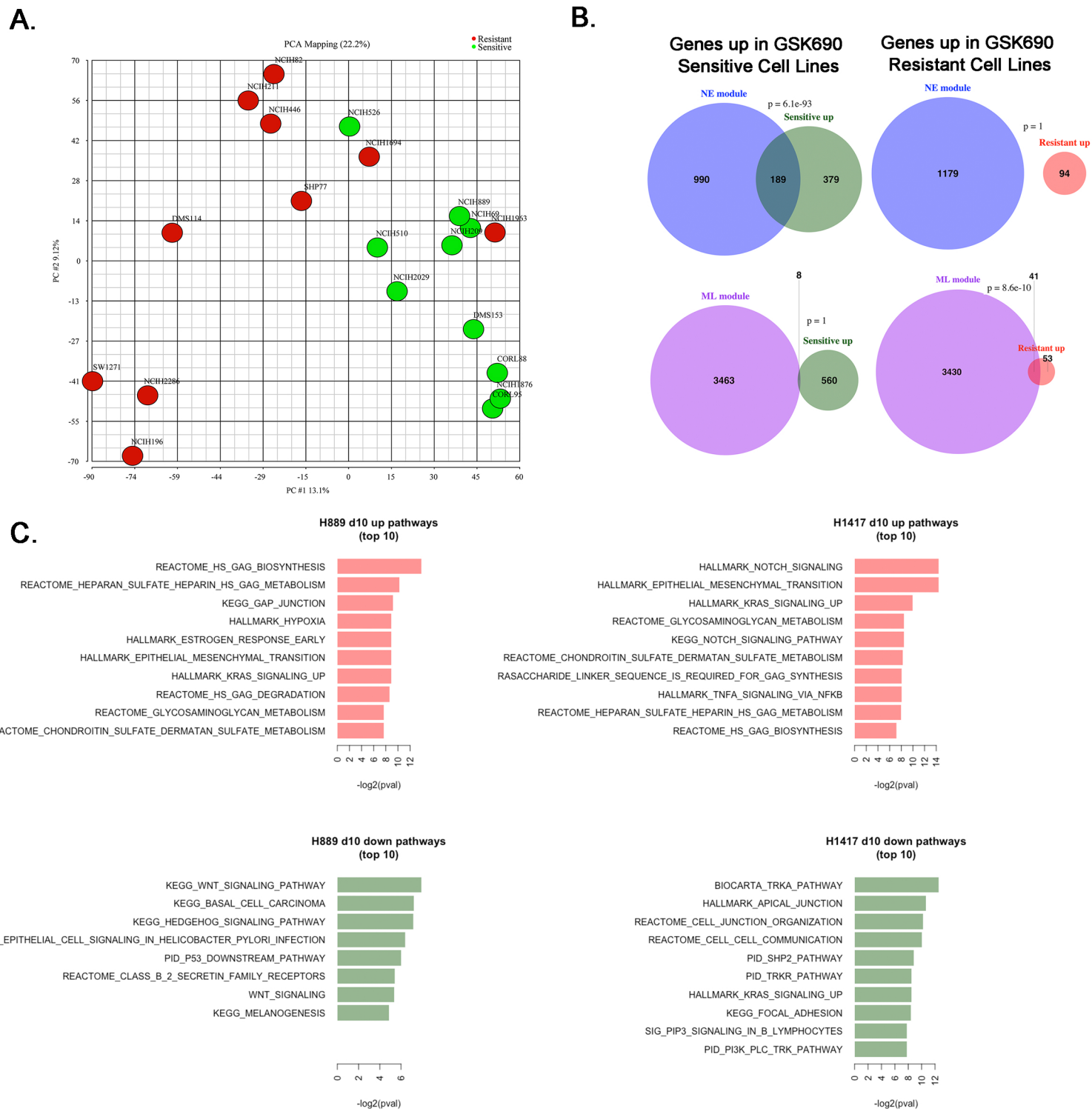

A.
PCA Mapping (22.2%)
Resistant
Sensitive
NCIH82
NCIH211
NCIH446
NCIH526
NCIH1694
SHP77
NCIH889
NCIH69
NCIH1963
DMS114
NCIH209
NCIH510
NCIH2029
DMS153
CORL88
SW1271
NCIH2286
NCIH1876
CORL95
NCIH196
PC #2 9.12%
PC #1 13.1%
B.
Genes up in GSK690 Sensitive Cell Lines
NE module
p = 6.1e-93
Sensitive up
990
189
379
Genes up in GSK690 Resistant Cell Lines
NE module
p = 1
Resistant up
1179
94
ML module
8
p = 1
Sensitive up
3463
560
ML module
41
p = 8.6e-10
Resistant up
53
3430
C.
H889 d10 up pathways (top 10)
REACTOME_HS_GAG_BIOSYNTHESIS
REACTOME_HEPARAN_SULFATE_HEPARIN_HS_GAG_METABOLISM
KEGG_GAP_JUNCTION
HALLMARK_HYPOXIA
HALLMARK_ESTROGEN_RESPONSE_EARLY
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION
HALLMARK_KRAS_SIGNALING_UP
REACTOME_HS_GAG_DEGRADATION
REACTOME_GLYCOSAMINOGLYCAN_METABOLISM
REACTOME_CHONDROITIN_SULFATE_DERMATAN_SULFATE_METABOLISM
0 2 4 6 8 10 12
-log2(pval)
H1417 d10 up pathways (top 10)
HALLMARK_NOTCH_SIGNALING
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION
HALLMARK_KRAS_SIGNALING_UP
REACTOME_GLYCOSAMINOGLYCAN_METABOLISM
KEGG_NOTCH_SIGNALING_PATHWAY
REACTOME_CHONDROITIN_SULFATE_DERMATAN_SULFATE_METABOLISM
RASACCHARIDE_LINKER_SEQUENCE_IS_REQUIRED_FOR_GAG_SYNTHESIS
HALLMARK_TNFA_SIGNALING_VIA_NFKB
REACTOME_HEPARAN_SULFATE_HEPARIN_HS_GAG_METABOLISM
REACTOME_HS_GAG_BIOSYNTHESIS
0 2 4 6 8 10 12 14
-log2(pval)
H889 d10 down pathways (top 10)
KEGG_WNT_SIGNALING_PATHWAY
KEGG_BASAL_CELL_CARCINOMA
KEGG_HEDGEHOG_SIGNALING_PATHWAY
EGG_EPITHELIAL_CELL_SIGNALING_IN_HELICOBACTER_PYLORI_INFECTION
PID_P53_DOWNSTREAM_PATHWAY
REACTOME_CLASS_B_2_SECRETIN_FAMILY_RECEPTORS
WNT_SIGNALING
KEGG_MELANOGENESIS
0 2 4 6
-log2(pval)
H1417 d10 down pathways (top 10)
BIOCARTA_TRKA_PATHWAY
HALLMARK_APICAL_JUNCTION
REACTOME_CELL_JUNCTION_ORGANIZATION
REACTOME_CELL_CELL_COMMUNICATION
PID_SHP2_PATHWAY
PID_TRKR_PATHWAY
HALLMARK_KRAS_SIGNALING_UP
KEGG_FOCAL_ADHESION
SIG_PIP3_SIGNALING_IN_B_LYMPHOCYTES
PID_PI3K_PLC_TRK_PATHWAY
0 2 4 6 8 10 12
-log2(pval)

Figure S5

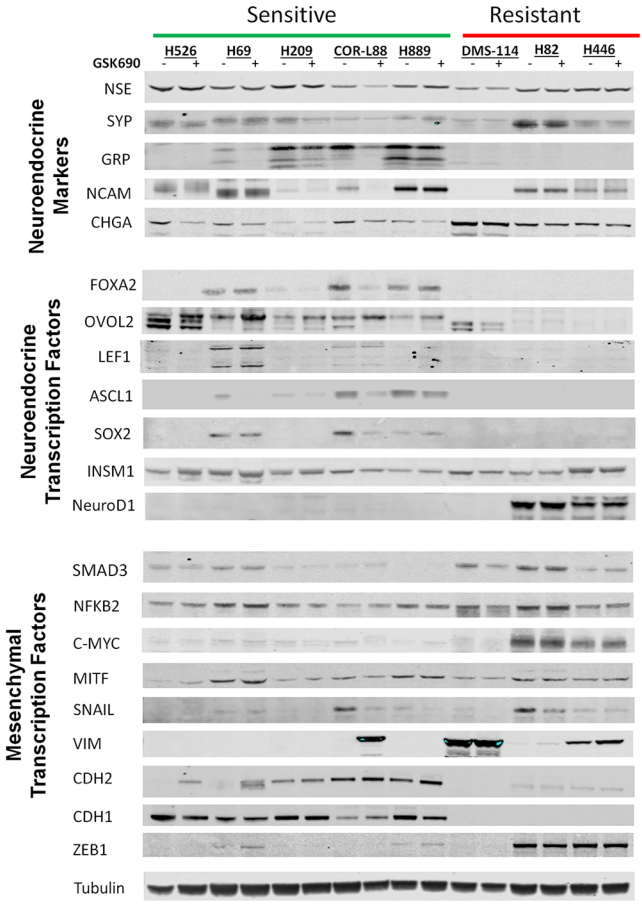
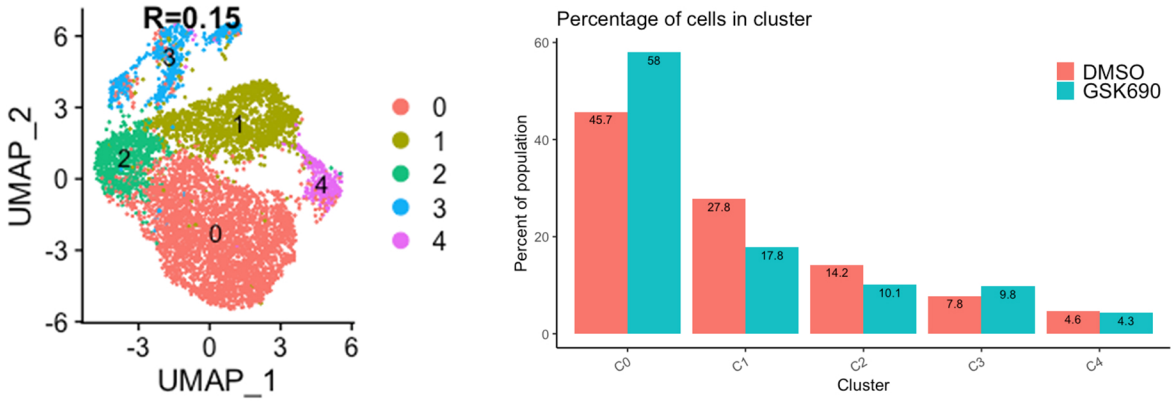

Sensitive
Resistant
H526
H69
H209
COR-L88
H889
DMS-114
H82
H446
GSK690
- + - + - + - + - + - + - + - +
Neuroendocrine Markers
NSE
SYP
GRP
NCAM
CHGA
Neuroendocrine Transcription Factors
FOXA2
OVOL2
LEF1
ASCL1
SOX2
INSM1
NeuroD1
Mesenchymal Transcription Factors
SMAD3
NFKB2
C-MYC
MITF
SNAIL
VIM
CDH2
CDH1
ZEB1
Tubulin

Figure S6

A.


R=0.15
UMAP_2
UMAP_1
0
1
2
3
4
Percentage of cells in cluster
Percent of population
Cluster
DMSO
GSK690
45.7
58
27.8
17.8
14.2
10.1
7.8
9.8
4.6
4.3
C0
C1
C2
C3
C4

B.

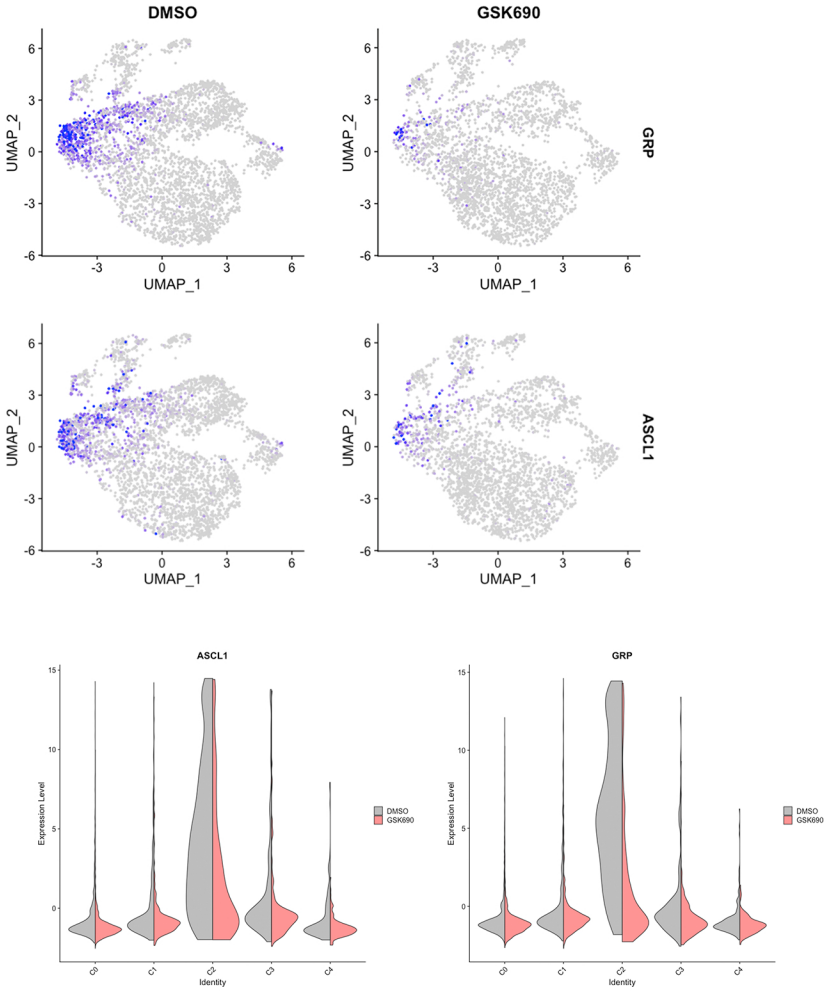
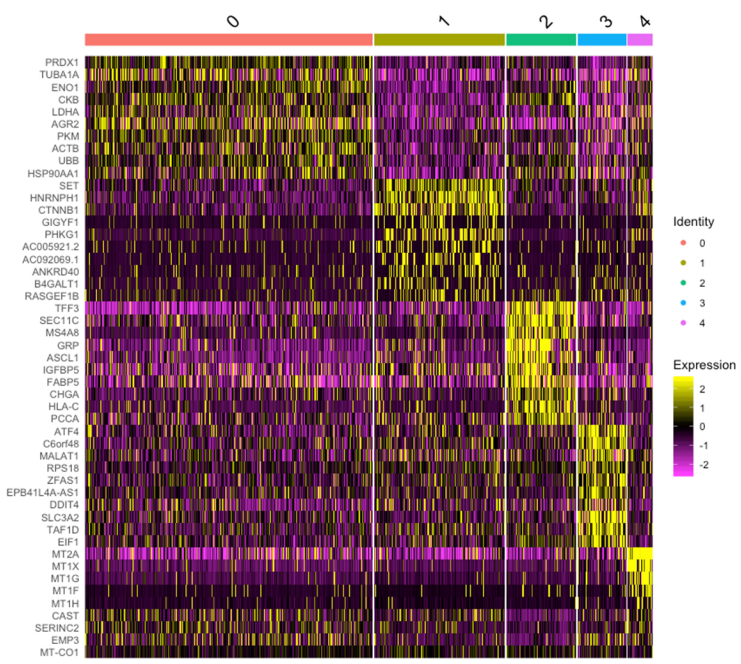

DMSO
GSK690
GRP
ASCL1
UMAP_1
UMAP_2
Expression Level
Identity
DMSO
GSK690

Figure S7

A.


Top 10 differentially expressed genes in each cluster
0
1
2
3
4
PRDX1
TUBA1A
ENO1
CKB
LDHA
AGR2
PKM
ACTB
UBB
HSP90AA1
SET
HNRNPH1
CTNNB1
GIGYF1
PHKG1
AC005921.2
AC092069.1
ANKRD40
B4GALT1
RASGEF1B
TFF3
SEC11C
MS4A8
GRP
ASCL1
IGFBP5
FABP5
CHGA
HLA-C
PCCA
ATF4
C6orf48
MALAT1
RPS18
ZFAS1
EPB41L4A-AS1
DDIT4
SLC3A2
TAF1D
EIF1
MT2A
MT1X
MT1G
MT1F
MT1H
CAST
SERINC2
EMP3
MT-CO1
Identity
0
1
2
3
4
Expression
2
1
0
-1
-2

B.

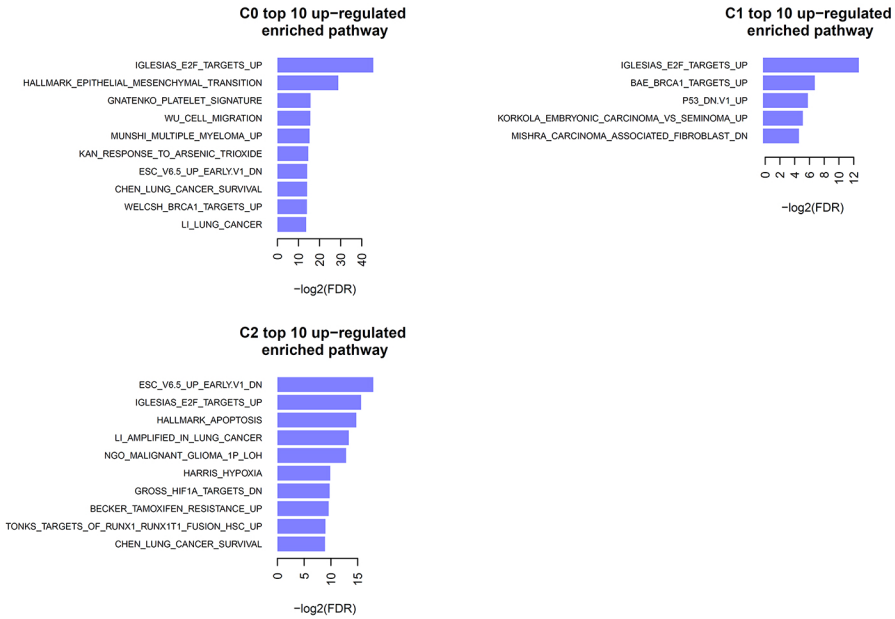
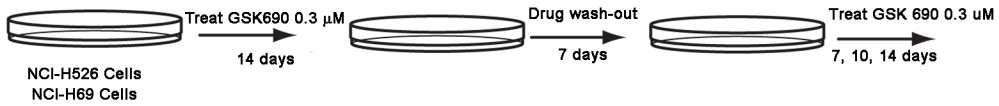
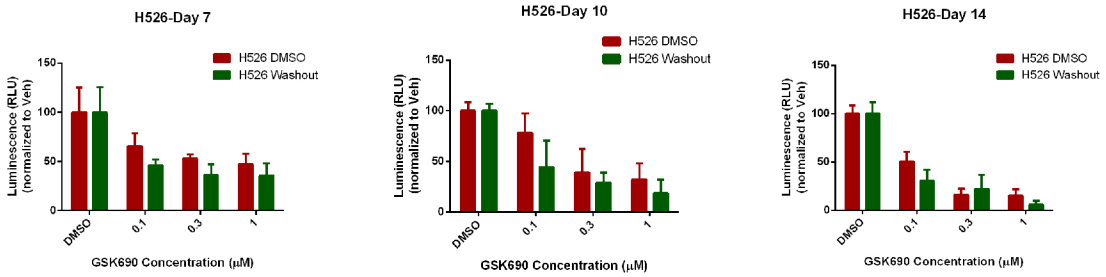
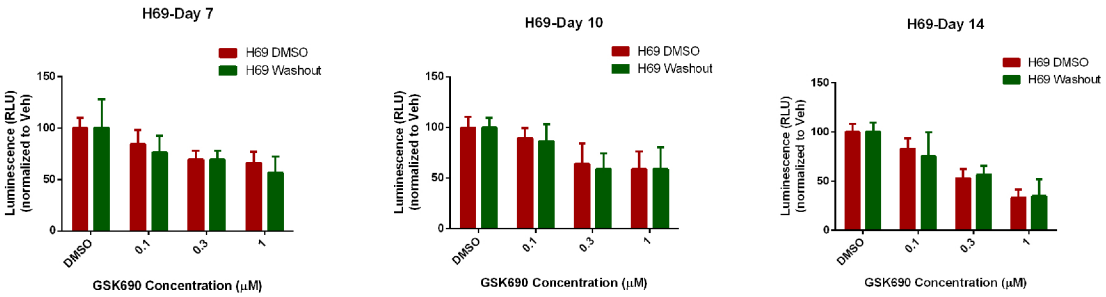
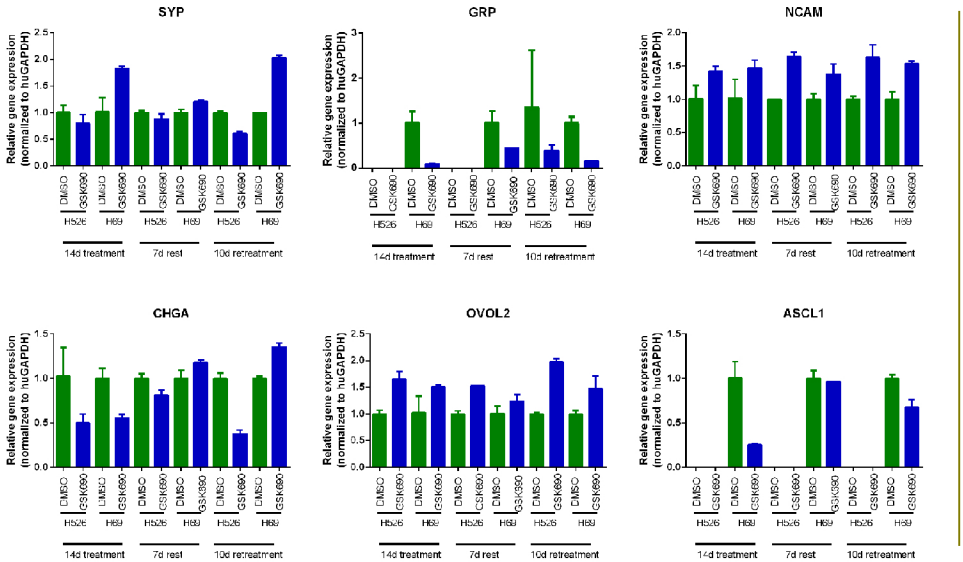

C0 top 10 up−regulated enriched pathway
IGLESIAS_E2F_TARGETS_UP
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION
GNATENKO_PLATELET_SIGNATURE
WU_CELL_MIGRATION
MUNSHI_MULTIPLE_MYELOMA_UP
KAN_RESPONSE_TO_ARSENIC_TRIOXIDE
ESC_V6.5_UP_EARLY.V1_DN
CHEN_LUNG_CANCER_SURVIVAL
WELCSH_BRCA1_TARGETS_UP
LI_LUNG_CANCER
0
10
20
30
40
−log2(FDR)
C1 top 10 up−regulated enriched pathway
IGLESIAS_E2F_TARGETS_UP
BAE_BRCA1_TARGETS_UP
P53_DN.V1_UP
KORKOLA_EMBRYONIC_CARCINOMA_VS_SEMINOMA_UP
MISHRA_CARCINOMA_ASSOCIATED_FIBROBLAST_DN
0
2
4
6
8
10
12
−log2(FDR)
C2 top 10 up−regulated enriched pathway
ESC_V6.5_UP_EARLY.V1_DN
IGLESIAS_E2F_TARGETS_UP
HALLMARK_APOPTOSIS
LI_AMPLIFIED_IN_LUNG_CANCER
NGO_MALIGNANT_GLIOMA_1P_LOH
HARRIS_HYPOXIA
GROSS_HIF1A_TARGETS_DN
BECKER_TAMOXIFEN_RESISTANCE_UP
TONKS_TARGETS_OF_RUNX1_RUNX1T1_FUSION_HSC_UP
CHEN_LUNG_CANCER_SURVIVAL
0
5
10
15
−log2(FDR)

Figure S8

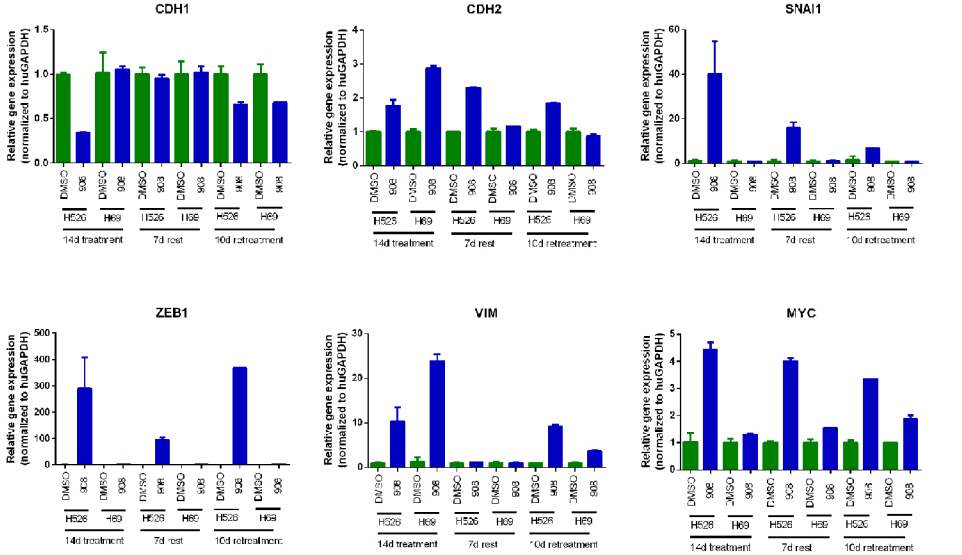








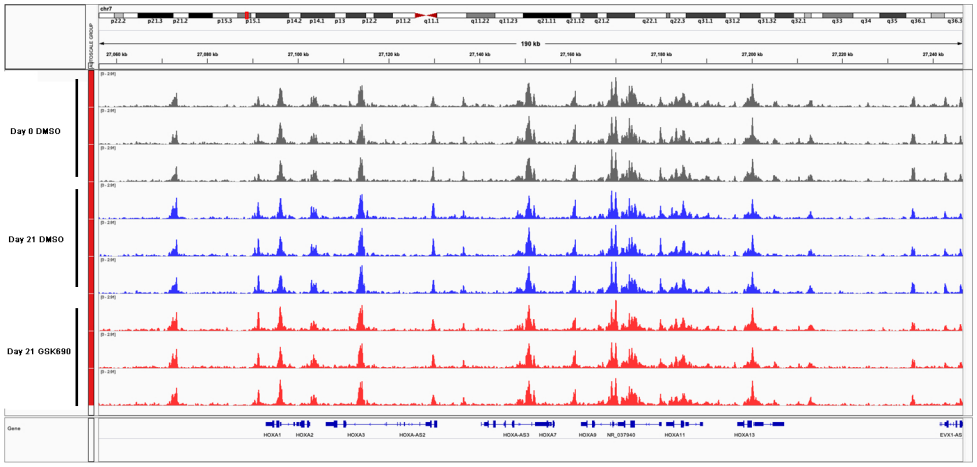
Neuroendocrine Markers



Mesenchymal Markers

Figure S9

A.



B.

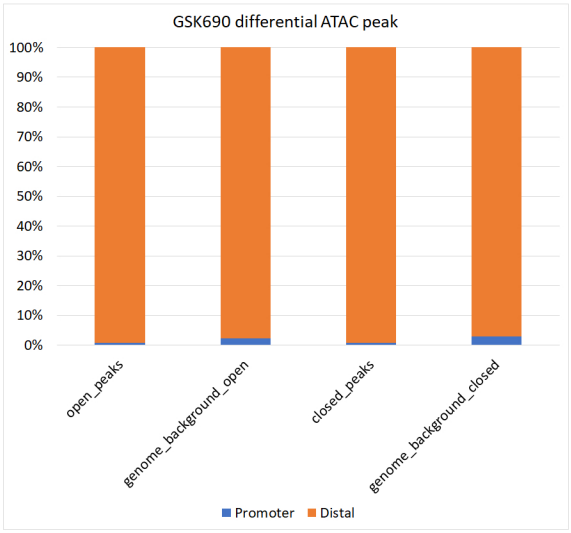


Figure S10